SPATIO-TEMPORAL VARIATION IN MACRO-INVERTEBRATE ASSEMBLAGE STRUCTURE OF KAYAR KHOLA, CHITWAN NEPAL

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Submitted to:

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Tribhuvan University

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September, 2022

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specially acknowledged by reference to the author(s) or institution(s).

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Date: 2022-08-26

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RECOMMENDATIONS

This is to recommend that the thesis entitled **"SPATIO-TEMPORAL VARIATION IN MACRO-INVERTEBRATE ASSEMBLAGE STRUCTURE OF KAYAR KHOLA, CHITWAN, NEPAL"** has been carried out by Sita Poudel for the partial fulfillment of Master's Degree of Science in Zoology with special paper **Fish Biology and Aquaculture.** This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor "**Prof Dr. Kumar Sapkota**, Central Department of Zoology, Tribhuvan University" this thesis submitted by Sita Poudel entitled "**SPATIO-TEMPORAL VARIATION IN MACRO-INVERTEBRATE ASSEMBLAGE STRUCTURE OF KAYAR, KHOLA CHITWAN, NEPAL**" is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by **Sita Poudel** entitled "**SPATIO-TEMPORAL VARIATION IN MACRO-INVERTEBRATE ASSEMBLAGE STRUCTURE OF KAYAR KHOLA, CHITWAN, NEPAL**" has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
APHA	American Public Health Association
BOD	Biological oxygen demand
$CaCO_3$	Calcium carbonate
CO_2	Free carbon-dioxide
DO	Dissolved Oxygen
EDTA	Ethylenediamine tetraacetic acid
EPA	Environmental Protection Agency
HCL	Hydrochloric acid
mg/l	Milligram per litre
NEPBIOS	Neplease Biotic Score
pH	Hydrogen ion Concentration
WHO	World Health Organization
WQI	Water Quality Index

ABSTRACT

The spatial and temporal variations of the macro-invertebrate assemblages in rivers and streams of Nepal are poorly understood. The macro-invertebrate communities and their relationships with environmental variables were studied in Kayar Khola, Chitwan for one year (November 2020 to August 2021) covering four seasons. Each and every sampling station was visited once in each season. Macro-invertebrate sampling was conducted at each sampling station from 11 am to 2 pm by using a D-frame Net with a mesh size of 0.5mm. Altogether 42 Families belonging to 17 Orders, 5 Classes and 3 Phyla were found in present study. Among the collected macro-invertebrates, Hydropsychidae (20.20%) was recorded to be the most dominant family and followed by Thiaridae (15.76%), Heptageniidae (12.02%), Baetidae (9.43%), and Psephenidae (5.09%) respectively. Family Viviparidae (0.05%) was less dominant and found only in the spring season at site C of Kayar Khola. The Order Trichoptera was found to be the most dominant order with 408 individuals. The highest Shannon-Weiner diversity index (2.39) was observed during winter season whereas the lowest value (1.61) observed in summer season. Maximum family richness value (21.0) was found in winter season and the minimum (7.75) found in summer season. The correspondence analysis (CCA) reveals a significant correlation between macro-invertebrate assemblage and environmental variables. Cluster analysis depicts that similarity between macro-invertebrates decreases as the distance of sites increases.

1. INTRODUCTION

1.1 Background

Nepal is abundant in freshwater resources, with over 6000 rivers and rivulets. The melting of snow is the source of the majority of Nepal's large and perennial rivers (Moog et al. 2008). Rivers occupy nearly 2.5% of the country's total land area. A variety of anthropogenic stresses such as eutrophication (Telesh et al. 1999), organic pollution (de Deckere et al. 2012), agricultural intensification (Dahal et al. 2007) and river bed extraction (Kondolf 1997) affect rivers worldwide, influencing river morphology and biodiversity. Headwater streams are often the only lotic habitats that have not been altered. They are extremely changeable settings with rich and unique biota (Malmqvist & Hoffsten 2000). A significant portion of the biodiversity of headwater streams is accounted for by macro-invertebrates.

Spatio-temporal refers to the relationship between space and time, with both spatial extension and temporal duration. The variation of anything relating to location and time is referred to as spatio-temporal variation. The majority of freshwater ecological studies have highly recommended biological monitoring of water bodies in addition to environmental features (Dudgeon 1999). A greater understanding of the significance of spatial and temporal variability in benthic macro-invertebrate populations is critical for enhancing our understanding of the variables impacting the structure and function of aquatic ecosystems, and, ultimately, aquatic biodiversity management.

Benthic macro-invertebrates are a crucial component of the river biota. They are small and spend at least part of their lives on the bottom of substrates such as sediments, debris, logs, and filamentous algae in streams, rivers, and lakes (Rosenberg 1993). And their sizes range between 200 to 500 µm (Ward 1998). According to Shah et al. (2011), macro-invertebrate research began in Nepal in the 1950s. The invertebrates that live in water and play an important part in aquatic ecology are macro-invertebrates or macro-zoobenthos. Many aquatic insects, according to aquatic biologists, developed in cool lotic water bodies before moving to warmer riverine and lacustrine environments (Hynes 1970). Benthic macro-invertebrates are abundant, have a relatively long life span, and are available as cost effective means of sampling measure (Rosenberg 1992).

They are affected by various environmental parameters operating at multiple spatio-temporal scales. Some of these factors include environmental variables (Collier et al. 1998), land use patterns (Helms et al. 2009), and sediment characteristics (Jones et al. 2012).

There are diverse groups of benthic macro-invertebrates. They include insects, bivalves, gastropods, annelids and crustaceans. They usually live in the stream bed sediments of rivers, lakes, and oceans and they feed on algae, bacteria, and leaves, as well as other organic matter in water (Xu et al. 2012). In freshwater ecosystems, insect larvae constitute the most abundant category among macro-invertebrates. Depending on the type of the habitat available, different types of macro-invertebrates are found. Mayflies, and caddisflies are generally intolerant of pollution whereas worms, midges and some molluscs are tolerant to pollution (Alam et al. 2008). Pollution intolerant organisms are found in good quality water and pollution tolerant organisms are found in bad quality water. Other macro-invertebrates, such as stoneflies, alderflies, and dobsonflies, are sensitive to pollution and can only be found in clean rivers. Macro-invertebrates are considered the best biological indicators of freshwater quality since they are impacted by changes in river natural variables such as width, depth, type of substratum, water velocity, and other physicochemical variables (Baptista et al. 2007). It is well acknowledged that macrofauna respond to hydraulic, organic, and toxic stress by reducing sensitive species and increasing the number of tolerant ones (Verschut et al. 2015).

Macro-invertebrates are essential components of any aquatic environment because they constitute the foundation of the trophic level. Some of these biota serve as fish food, while others prey on young fish and other aquatic species. They also play a crucial role in nutrient cycle and energy transfer. Furthermore, aquatic invertebrates aid in the cleaning of rivers by utilizing organic and detritus matter, and assist in the assessment of the overall health of the stream and river (Carlisle et al. 2007).

Several biologists have outlined the advantages and disadvantages of employing benthic macro-invertebrates in biomonitoring. Benthic macro-invertebrates offer significant advantages over other aquatic organisms. They are not only abundant and have a reasonably long life cycle, but they are also extremely sensitive to changes in aquatic biological circumstances (Calapez et al. 2017). There are a number of drawbacks to employing benthic macro-invertebrates as biomonitoring tools (Hawkes 1979). For example, it is difficult to sample quantitatively and identification of some taxa is time consuming and requires experts.

Many biologists have conducted research on water macro-invertebrate species (Das 1971). Some researchers investigated their abundance by computing diversity indices as biomonitors and indicators of water quality and environmental conditions in lotic water bodies (Norris & Morris 1995). Some works have also been done in Nepalese water bodies (Sharma et al. 2005). However, there is no any research regarding macro-invertebrates conducted on Kayar Khola, Chitwan, Nepal. Kayar Khola originates in the Mahabharat hills of North Chitwan and ultimately drains to the East Rapti at the Khorsor zone of the Barandabhar corridor in Chitwan National Park. The Rapti River flows from north-east to south-west and ultimately mixes with the Narayani River System.

1.2 Objectives

1.2.1 General objective

- To investigate the spatio-temporal variation in macro-invertebrate assemblage structure of Kayar Khola, Chitwan, Nepal.

1.2.2 Specific objective

- To explore the spatial variation of macro-invertebrate assemblage structure.
- To find out the temporal variation of macro-invertebrate assemblage structure.
- To describe the relationship between environmental variables and macro-invertebrate assemblage structure.

1.3 Rationale of the study

The spatial and temporal variations of the macro-invertebrate assemblages in rivers and streams of Nepal are poorly understood. Therefore, research into the geographical and temporal variation of macro-invertebrate assemblages in rivers and streams of Nepal is required to better monitor, manage, conserve, and understand their status. As these organisms are the best indicators for controlling water pollution and regulating water bodies to prevent future deterioration, they must be protected. Despite their importance, Kayar Khola has not yet been largely investigated and its biodiversity is still unknown by the scientific world, so I have chosen this river system. This research will provide information on the spatial and temporal variance of macro-invertebrate assemblages in Kayar Khola. The data obtained from this study was baseline data for further study. Furthermore, taxonomic documentation of these species is required to contribute to and maintain the aquatic biodiversity database. Hence, the research on these aspects are essential**.**

1.4 Research questions

How does the macro-invertebrate assemblage change along the spatial and temporal gradient of the river?

What environmental variables are associated with spatial and temporal changes in assemblage structure?

1.5 Limitations and Delimitations

- The study only covered four seasons (i.e Winter, Spring, Summer & Autumn), not each month.
- All the aquatic macro-invertebrates were identified up to family level.

2. LITERATURE REVIEW

Rivers support a wide range of creatures, including diatoms, insects, fish, reptiles, and mammals. River water fulfills the primary needs (drinking, cleaning, irrigation, and hydro-electricity generation) of people in both the rural and urban areas. The flow of water, nutrients, inorganic and organic elements from headwater mountain streams to lowland rivers is unidirectional in the river ecosystem (Suren 1994). Macro-invertebrates that rely on these substances are extremely sensitive to temperature, precipitation, and stream flow regime (Bunn & Arthington 2002).

2.1 Spatial and temporal distribution of Macro-invertebrates

Benthic macro-invertebrates are bottom-dwelling organisms. They are large enough to be seen with the naked eye and retained on a 0.5 mm mesh net. These invertebrates are present in both lotic and lentic habitats, often dwelling among the rocks and sediments. Freshwater rivers, streams, marshes, and lakes are the areas where macro-invertebrates are found the most. These aquatic organisms include insects, bivalves, gastropods, annelids, and crustaceans. Furthermore, these species are extremely vulnerable to water sediment degradation caused by physical or chemical stressors (Alam et al. 2008).

Macro-invertebrates are significant components of riverine systems due to their tremendous taxonomic and functional diversity (Malmqvist 2002). They are found on the substrata of an aquatic habitat (Dernie et al. 2003). These organism's abundance and distribution are affected by habitat conditions and other environmental factors (Cyr & Downing 1988). Mehler et al. (2015) found that the composition of benthic macro-invertebrate communities changed spatially and temporally in response to the physicochemical environment. This study shows benthic macro-invertebrate diversity associated positively with substrate size and stream width but adversely with stream temperature and organic contaminants. Aquatic macro-invertebrate diversity and composition in Southeastern Brazil streams along an altitudinal gradient was observed by Henriques-Oliveira & Nessimian (2010). According to them, the highest richness was found at elevations of 1200-1300 m, while the lowest was found in altitudes below 100 m.

Canopy cover and environmental factors affected macro-invertebrates abundance, diversity, and richness and that the individual taxon had varying responses to these factors (Arimoro et al. 2012). They found that the river hosted a diverse macro-invertebrates, with Decapoda, Ephemeroptera, Odonata, Gastropoda, Trichoptera, and Coleoptera dominating upstream sample stations with extensive canopy cover, and Diptera and Coleoptera dominating downstream sampling stations with less canopy cover. The spatiotemporal fluctuations of macro-invertebrates in the Mardi and Vijayapur streams were reported by Pokharel (2013) from Pokhara, Nepal. During the study period, 47 Genera (32 from the riffles and 34 from the pools) belonging to 38 Families and 12 Orders were identified (Pokhrel 2013). Sharma et al. (2005) investigated the effects of dams on macro-invertebrates in the Tinau River and identified 2120 macro-invertebrates representing 22 Families under 10 Orders. A study on macro-invertebrate diversity and water quality measures was carried out in the Godavari River of Nepal. According to Vaidya (2019), total 1 Phylum, 2 Classes, 6 Orders, 25 Families, and 1558 individuals were recorded from Godavari River with large abundances of Hydropsychidae, Baetidae, and Chironomidae. Shrestha & Adhikari (2016) found 2166 individual benthic macroinvertebrates from 10 Families and 7 Orders in Taudaha Lake, Kathmandu. Yadav & Rajbhandari (1982) investigated the benthic macrofauna of Bansbari khola and Dhobi khola in Kathmandu valley. They recorded 26 taxa at various locations along both streams.

Benthic macro-invertebrate communities respond differently at different altitude levels. With the increase in altitude, the taxonomic richness decreases (Brewin et al. 1995). The impact of altitude and land-use changes on macro-invertebrate assemblages was recorded from riffles of forty-three streams in the Dolpo region of western Nepal (Suren 1994). The diversity differed significantly across the altitudinal range was observed by Bhandari et al. (2018) in tributaries of Buddhiganga River, Western Nepal. Macro-invertebrates from river systems in east- central Nepal demonstrated altitudinal alterations in assemblage composition (Ormerod et al. 1994). Sen (2010) conducted a baseline survey in Rampur Ghol to examine the general condition of ghol. In this research, the health of the ghol area was found to be in good condition and its water quality was not polluted by any natural sources. Changes in air and water temperature affect the growth and timing of the development of macro-invertebrates (Bayoh &

Lindsay 2003). Environmental changes may also affect the abundance and composition of benthic invertebrates.

2.2 Relation between physico-chemical parameters with macro-invertebrates

The composition of water is affected by various physical and chemical factors. Such factors include temperature, pH, dissolved oxygen, free carbon dioxide, total dissolved solid matter, hardness of water, alkalinity, etc. Any changes in these factors can have an impact on the aquatic biota. Water quality assessment of major rivers in Nepal was carried out by Pradhan (1998). These studies reported the information regarding benthic invertebrate fauna on the effects of natural and anthropogenic effects from Nepal. The macro-invertebrates were used as economic tools for determining water quality of Bhalu khola, a tributary of Budhigandaki River as efficient water quality indicators (Rana & Chhetri 2015). According to this study, a total of 103 macro-invertebrates from 11 Families and five Orders were discovered. Similarly, the water quality index (WQI) and the NEPBIOS index revealed the same result, indicating that the water quality was good. The effect of several environmental conditions on benthic macro-invertebrate communities in the Bagmati River headwaters was examined by Rai et al. (2019). The West Seti River, a tributary of the Karnali in West Nepal, was tested for water quality using macro-invertebrates as biological indicators (Matangulu et al. 2017). Dahal et al. (2007) investigated the effects of agricultural intensification on river quality in rural watersheds of Nepal, which reveals physical parameters like temperature and pH are less susceptible to agricultural land use. Popoola & Otalekor (2011) observed that pH and temperature are negatively correlated, Dissolved oxygen (DO) had an inverse association with pH and temperature while alkalinity, and velocity had a direct correlation with DO. The spatial distribution of benthic macro-invertebrate assemblages in relation to environmental variables, particularly pollution level, has been described from Korean nationwide streams (Jun et al. 2016).

EPA (2008) reported that water temperature plays an important role in maintaining aquatic macro-invertebrate biological activities (such as growth, metabolic rates, reproduction, and survival). Daufresne et al. (2004) found that spring macro-invertebrates are decreased with the increase in temperature. According to this study, the temperature rose by 1°C over a 25-year period, and the abundance of macro-invertebrates decreased by around 21%.

Availability of DO is an important factor for composition and distribution of freshwater communities (Hynes 1960). The concentration of DO depends on multiple factors such as temperature, pressure, photosynthesis carried by aquatic plants, respiration by aquatic organisms (Allan 1995). Connolly et al. (2004) reported that both upland and lowland stream macro-invertebrates responded similarly to DO content. Mayflies were the most sensitive to low oxygen conditions, and several highland and lowland species experienced lethal effects at DO levels of 20% saturation.

3. MATERIALS AND METHODS

3.1 Study area

Kayar Khola watershed is located in the northeastern part of Chitwan district, Nepal. It originates in the Mahabharat hills of North Chitwan and ultimately drains to the East Rapti at the Khorsor zone of the Barandabhar corridor in Chitwan National Park (Figure 1). The study was carried out from Chisapani, Shaktikhor at 27°42'26.01"N 84°34'37.24"E to Sauraha at 27°35'27.09"N 84°29'36.29"E. The total study area is 20 km long from station I to station IV. The Rapti River flows from north-east to south-west and ultimately mixes with the Narayani River System. Rapti River is remarkably helping the livelihood of people in East- Chitwan.

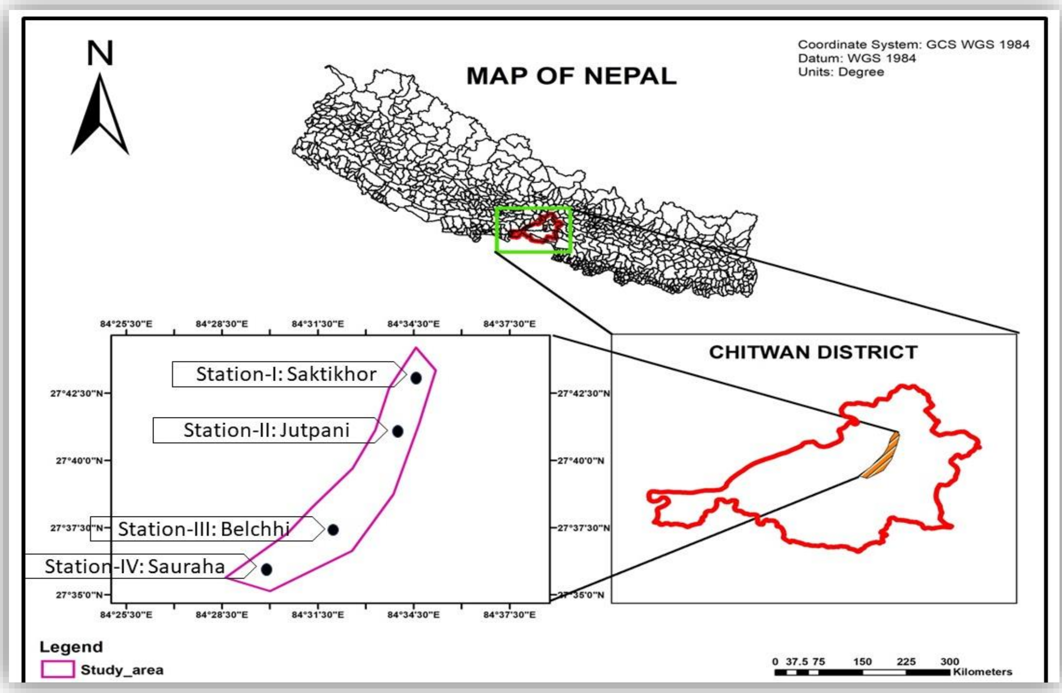

Figure 1: Map showing study area of Kayar Khola

3.2 Study period

The field work was conducted for one year (November 2020 to August 2021) covering four seasons. Four seasonal data were taken in November for autumn, January for winter, April for spring and August for summer. Each and every sampling station was visited once in each season.

3.3. Sampling Station Selection

Before beginning the study work, a preliminary survey was done for fixing the study sites. Sampling stations were selected based on the altitudinal variation, human settlement area, buffer zone, agricultural field area etc. Selected four sampling stations are given below:

Site A

Chisapani, Saktikhor is located in Kalika Municipality of Chitwan district and it is about 13 km from Bakullar chok, Tandi. It lies on 27°43'10.39"N 84°35'30.97"E and it has an elevation of 344m from sea level. There is a human settlement on the left side of the bank of the river. Mainly this area consists of hard bottom substrate. Gravel, cobble and boulder particles are 60% surrounded by fine sediment. Right bank was stable with native vegetation cover whereas the left bank was slightly disturbed.

Site B

Jutpani lies at Kalika Municipality of Chitwan district and 7 km downward from station I. It lies on 27°40'58.58"N 84°33'52.73"E and it has an elevation of 264m from sea level. Approximately 70% of substrate was suitable for the epifaunal colonization. Gravel, cobble and boulder were surrounded by fine sediment around 60%. Generally all flat water or shallow riffles were observed and less covered by native vegetation.

Site C

Belchi is located in Ratnanagar municipality. This station lies on 27°36'55.87"N 84°31'53.30"E and it has an elevation of 198m from sea level. Stone, gravel and cobble particles were around 20% surrounded by fine sediment. Right bank was not affected by any human activities but minimal human impact was noticed along the left bank. About 90% of this area is covered by vegetation.

Site D

Site D is located in Ratnanagar municipality. Sauraha lies on 27°35'27.09"N 84°29'36.29"E and it has an elevation of 187m from sea level. This station is near

Khorshor and comes under the buffer-zone area. Mainly this area consists of hard bottom substrate. Stone, gravel and cobble particles were surrounded by fine sediment around 40%. Right side of this bank is the grazing area and the left side is the human settlement area.

3.4 Materials

Materials like D-frame net, sieve, polythene bags, vials, stereo microscope, thermometer, pH meter, BOD bottle were used during the field and lab work.

3.5 Macro-invertebrate Sampling and identification

Four sites were chosen along the river based on accessibility, closeness to pollution sources, and the availability of historical records from previous water quality studies to allow for a comparative study of long-term temporal changes. It is necessary to sample each site several times a year at regular intervals to minimize the chances of taxa being missed. Macro-invertebrates were collected at each site by using a D-frame Net of mesh size 0.5mm and the sampling was done at 11am to 2pm in every sampling station. Sampling was conducted within 500 m. Collected samples were screened using the various mesh sieves and photographs were taken immediately using an iphone 7+ cellphone before the preservation of Macro-invertebrates. After photography, the collected macro-invertebrates were preserved in 70% alcohol and carried to the laboratory of the Central Department of Zoology for identification. Then the samples were sorted group-wise and identified to Family level on morphologically with the help of stereo microscope (10X4.5x magnification) by using available keys (Dudgeon 1999; Budha 2016; Shah et al. 2020).

3.6 Analysis of physico-chemical parameters of water

The various physico-chemical parameters of water were analyzed to know the water quality based on different factors which are temperature, pH, velocity, depth, dissolved oxygen, free carbon dioxide, total alkalinity and total hardness.

3.6.1 Physical parameters

3.6.1.1 Water temperature

Temperature of the water was taken by using a standard mercury thermometer. The bulb of the thermometer was dipped inside the surface of water and reading was taken. The result was expressed in Degree Celsius.

3.6.1.2 Water depth

Water depth was calculated using a stick which was already measured by a standard ruler and marked on its surface. Stick was dipped in three different places on the water and when it got bottom, it was noted down. Mean depth of three places was calculated.

3.6.1.3 Velocity

Water velocity of Kayar Khola was measured with the help of a stopwatch, a floating box and measuring tape. The result was measured in m/s.

3.6.2 Chemical parameters

3.6.2.1 Hydrogen ion Concentration (pH)

pH is the most important environmental factor influencing the composition and distribution of all aquatic creatures. A digital pH meter was used to record the hydrogen ion concentration of water during the study period at every station of the Kayar Khola

3.6.2.2 Dissolved Oxygen (DO)

Winkler's method was used to calculate the dissolved oxygen in water. To measure Dissolved Oxygen, the sample of every station was taken in a 300 ml BOD bottle by avoiding the air bubbles. Then the two millimeters of manganese sulphate and the same quantity of alkaline – iodine – azide (KI) solution was added well by the help of pipette to fix the Dissolved Oxygen. After fixing Dissolved Oxygen, let the bottle stay as it is for half an hour and titrated against sodium thiosulphate of 0.25N strength just after unfixing Dissolved Oxygen by adding conc. H_2SO_4 solution and starch indicator.

Dissolved Oxygen was calculated by using following formula:

$$DO\ (mg/l) = \frac{(ml\ \times N) of\ Na_2SO_3\ \times\ 44\ \times 1000}{\frac{V_2\ (V_1 - V)}{V_1}}$$

Where,

V= Volume of $MnSO_4$ and KI added

V_1=Volume of BOD bottle

V_2=Volume of the part of the content titrated

3.6.2.3 Free Carbon-dioxide (CO_2)

To determine the free CO_2, 50 ml of water sample was taken and a few drops of phenolphthalein indicator were added. The obtained colorless solution indicated the availability of carbon dioxide present in the sample. After that the solution was titrated against 0.05N sodium hydroxide (NaOH) to the faint pink end point. Level of free CO_2 in the water sample was calculated by the following equation.

Free CO_2 (as mg/l) = $\frac{(\text{ml} \times \text{N})\text{ of NaOH} \times 44 \times 1000}{V}$

Where,

V=Volume of water sample taken (ml)

3.6.2.4 Total Hardness

The total hardness of the river water was estimated by EDTA Titrimetric Method. For the calculation, 50 ml of water sample was taken in a conical flask. After that 2ml of ammonia buffer solution and 200 mg of Eriochrome Black – T indicator was added and mixed thoroughly by shaking the flask until the wine red color appeared. Then the solution was titrated against the standard EDTA solution (0.01N) till the clear blue colour was resolved. The total hardness was calculated as follows:

Total Hardness (mg/l as $CaCo_3$) = $\frac{\text{Vol.of EDTA used in ml} \times 1000}{V}$

Where,

V=Volume of water sample taken (ml)

3.6.2.5 Alkalinity

To determine alkalinity, 100 ml water sample was taken and 2 drops of phenolphthalein indicator were added. When the solution remains colorless, Phenolphthalein Alkalinity (PA) is zero. After adding Phenolphthalein, the color changed to pink, which was titrated with 0.1N HCL until the color disappeared at the end point. This is PA. After that 2-3 drops of methyl orange were added to the same sample and the titration was repeated until the yellow color turned to pink at the end point. This is total alkalinity (APHA 1998). They are calculated as follows:

$$\text{PA as } CaCO_3\text{, mg/l} = \frac{\text{A} \times \text{N of HCL} \times 1000 \times 50}{V}$$

$$\text{TA as } CaCO_3\text{, mg/l} = \frac{\text{B} \times \text{N of HCL} \times 1000 \times 50}{V}$$

Where,

A= ml of HCL used with phenolphthalein

B= ml of total HCL used with methyl orange and phenolphthalein

N= Normality of HCL used

PA= Phenolphthalein Alkalinity

V= Volume of sample used (ml)

TA= Total Alkalinity

3.7 Data Analysis

3.7.1 Species Diversity

Shannon-Weiner diversity index (Shannon and Weaver, 1949) was designated as H', which was calculated as:

$$H' = -\Sigma\ (ni\ /N) \log (ni/N)$$

Where,

ni = Importance values for each species is the number of individuals in each species, the abundance of each species.

N = Total importance value, the total number of individuals observed.

3.7.2 Species richness index

The species richness was calculated by using Margalef Species richness (Margalef's 1968). Margalef richness index is designated by d, and calculated mathematically as:

Here, species richness (d) = S-1/ log N

Where, S= Number of species

N = Number of individuals

3.7.3 Evenness index

The evenness of the species was calculated by using Pielou's species evenness (Pielou 1996). To calculate whether species are distributed evenly across seasons and across landscapes elements, the evenness index was determined by using the following equation.

E= H'/ log S

Where,

H' = Shannon-Wiener's diversity index.

S= Species richness is the number of species and is simply a count of the number of different species in a given area.

3.8 Statistical Analysis

3.8.1 One-way ANOVA (analysis of variance) test

The relationship between diversity indices of macro-invertebrate and seasons were analyzed by using R programme.

3.8.2 Cluster analysis

Macro-invertebrate taxa were analyzed into different assemblage clusters based upon abundances of each macro-invertebrate by utilizing pvclust package in R (Hubbard et al. 2019).

3.8.3 Multivariate analysis

The relation between macro-invertebrate taxa and environmental variables were analyzed by Canonical Correspondence analysis (CCA) method (Ter Braak 1986) based on a linear response of species to environmental gradients was applied by using vegan library in R (Oksanen et al. 2013).

4. RESULTS

4.1 Spatial and temporal Variation of macro-invertebrate assemblage structure

4.1.1 Distributional pattern and frequency occurrence of macro-invertebrates

Altogether 42 Families belonging to 17 Orders, 5 Classes and 3 Phyla were found in this study. The distribution patterns and frequency occurrence of various species of macro-invertebrates in Kayar khola is shown in ANNEX III. The Hydropsychidae family (20.20%), Thiaridae (15.76%), Heptageniidae (12.02%), Baetidae (9.43%), and Psephenidae (5.09%) were found higher frequency across all four stations and seasons. The Viviparidae (0.05%), Pyralidae (0.10%), Pachychilidae (0.15%), Glossiphoniidae (0.15%), Megascolecidae (0.15%), Ephydridae (0.15%), Naucoridae (0.15%), Aphelocheiridae (0.15%) and Philopotamidae (0.15%) families were found in lower frequency. Among the lowest distributions Viviparidae was found only in the spring season at site C of Kayar Khola.

Table 1: Diversity of macro-invertebrates in Kayar Khola

S. N	Phylum	Class	Order	Family	Scientific name
1	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	*Heptagenia* sp.
2				Baetidae	*Baetis* sp.
3				Ephemeridae	
4				Ephemerellidae	*Serratella* sp.
5				Caenidae	*Caenis* sp.
6			Trichoptera	Hydropsychidae	*Hydropsyche* sp.
7				Philopotamidae	
8			Coleoptera	Psephenidae	
9				Hydrophilidae	
10			Odonata	Chlorocyphidae	
11				Gomphidae	*Ophiogomphus* sp.

12				Corduliidae	
13				Libellulidae	
14				Coenagrionidae	
15				Cordulegastridae	
16				Calopterygidae	
17				Aeshnidae	
18			Hemiptera	Nepidae	
19				Gerridae	
20				Pleidae	
21				Aphelocheiridae	*Aphelocheirus* sp.
22				Naucoridae	
23			Diptera	Tabanidae	*Tabanus* sp.
24				Simuliidae	*Simulium* sp.
25				Culicidae	
26				Chironomidae	
27				Ephydridae	
2			Plecoptera	Perlidae	*Neoperla* sp.

8					
29			Megaloptera	Corydalidae	*Corydalus* sp.
30			Lepidoptera	Pyralidae	*Aulocodes* sp.
31		Malacostraca	Decapoda	Palaemonidae	
32				Potamidae	
33	Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	
34			Haplotaxida	Megascolecidae	
35			Arhynchobdellida	Salifidae	
36	Mollusca	Gastropoda	Caenogastropoda	Thiaridae	*Tarebia granifera* Lamarck, 1822
37					*Tarebia lineata* Gray, 1828
38					*Melanoides tuberculata* Mueller, 1774
39				Pachychilidae	*Brotia costula* Rafinesque, 1833
40			Basommatophora	Lymnaeidae	*Lymnaea acuminata* Lamarck, 1822
41				Planorbidae	*Gyraulus convexiusculus* Hutton, 1849
42				Bulinidae	*Indoplanorbis exustus* Deshayes, 1834

43			Mesogastropoda	Viviparidae	*Bellamy bengalensis* Lamarck, 1822
44		Bivalvia	Unionoida	Unionidae	*Parreysia* sp.
45					*Radiatula* sp.

4.1.2 Diversity status of macro-invertebrates fauna of Kayar Khola

The value of Shannon Weiner diversity index (H'), Family richness index (d) and Evenness index (E) were calculated based on stations and seasons (figures 2&3). The highest Shannon Weiner diversity index (2.46) was observed at site D and the lowest (1.88) was observed at site C. Similarly, the highest Shannon Weiner diversity index (2.39) was observed in winter season and the lowest value (1.61) was observed in summer season. Maximum family richness value (18.75) was found at site D whereas the minimum value (12.75) was found at station B. The highest family richness (21.0) was found in the winter season and lowest (7.75) in summer season. The highest Evenness index (0.87) was observed at station D and lowest (0.72) at station C. Similarly, maximum Evenness index (0.79) was found in winter and summer season whereas minimum (0.76) in spring season.

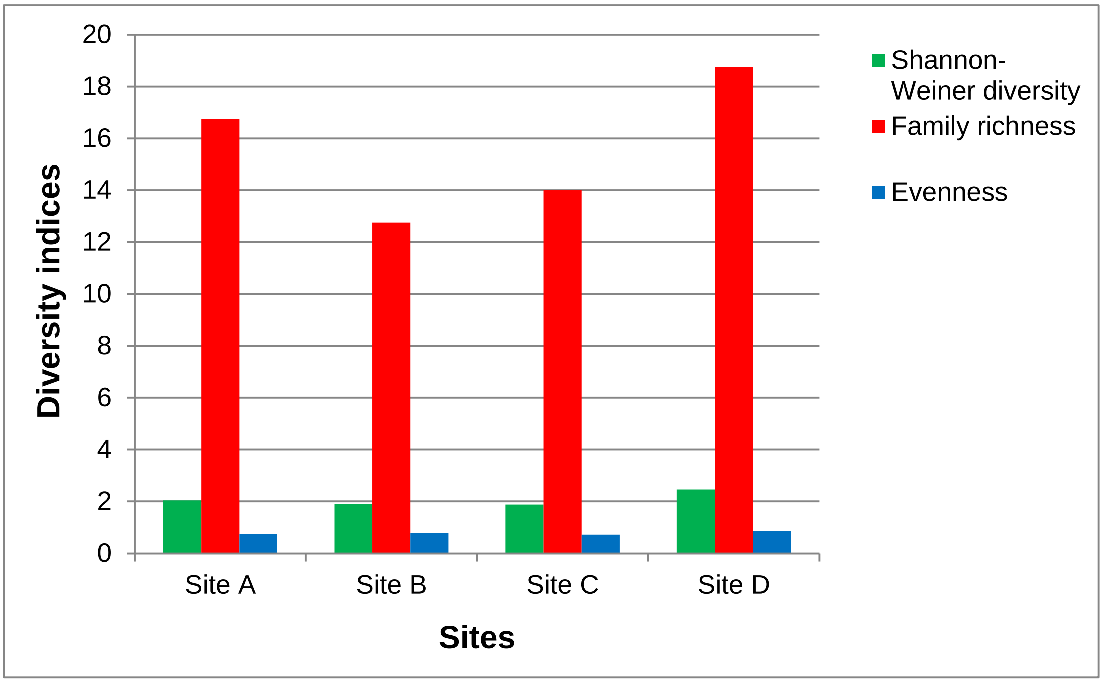

Figure 2: Spatial variation of macro-invertebrate diversity in Kayar Khola

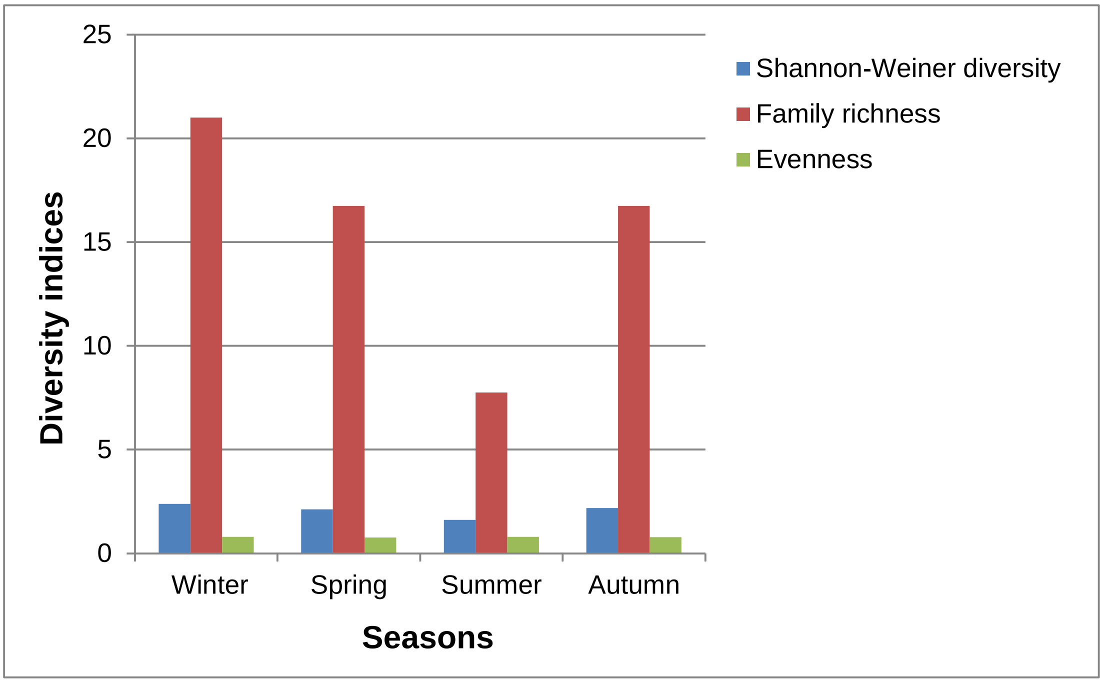

Figure 3: Temporal variation of macro-invertebrate diversity in Kayar Khola

4.2 Spatial and temporal variation of the environmental variables

4.2.1 Physical parameters of water

4.2.1.1 Water Temperature

The water temperature was found to be highest at site D (33.3°C) during the summer season and lowest at site A (22°C) during the winter season. The variation in temperature during different seasons at different sites were shown in figure 4.

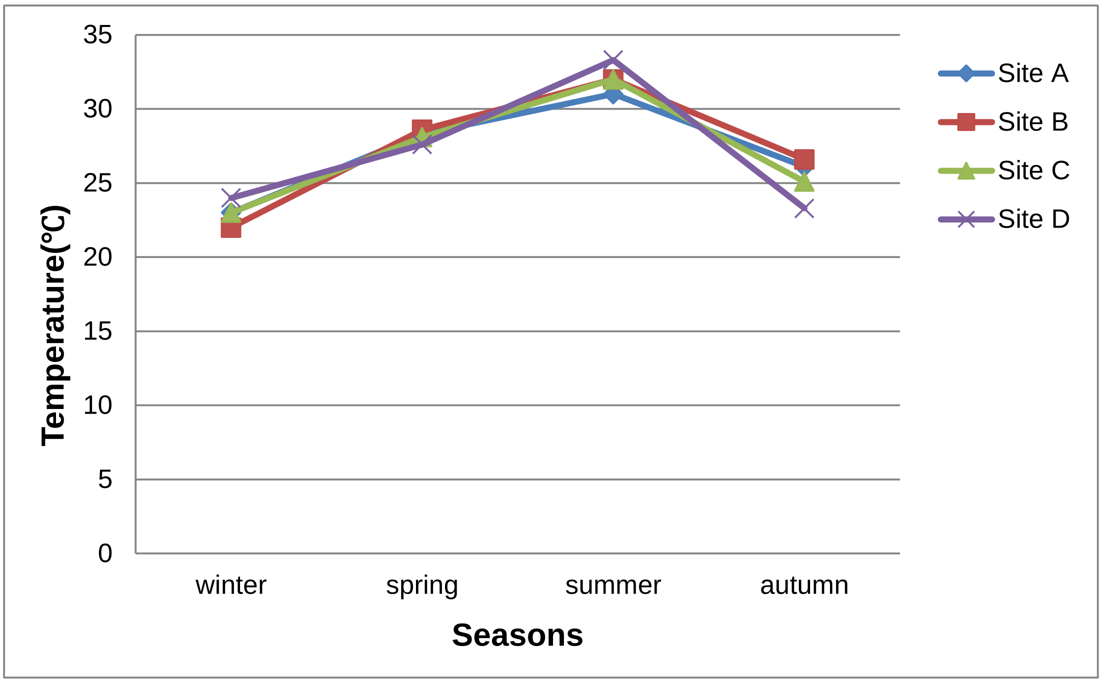

Figure 4: Variation of water temperature at different sampling sites and seasons

4.2.1.2 Water depth

The depth of Kayar khola varies from season to season. At site D during summer season, depth was found to be highest i.e. 1.3 m whereas it was recorded lowest at site A in winter season i.e. 0.26 m.

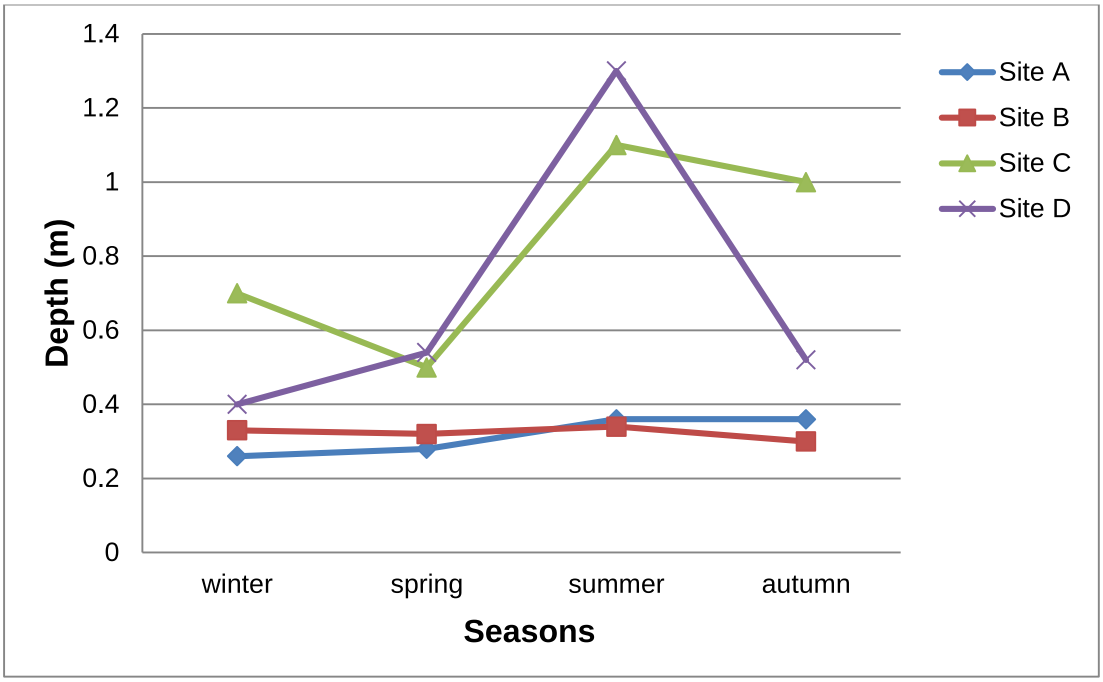

Figure 5: Variation of water depth at different sampling sites and seasons

4.2.1.3 Velocity

The velocity was found maximum at site A during summer season (0.93 m/s) whereas it was recorded minimum at site A in spring season (0.32 m/s).

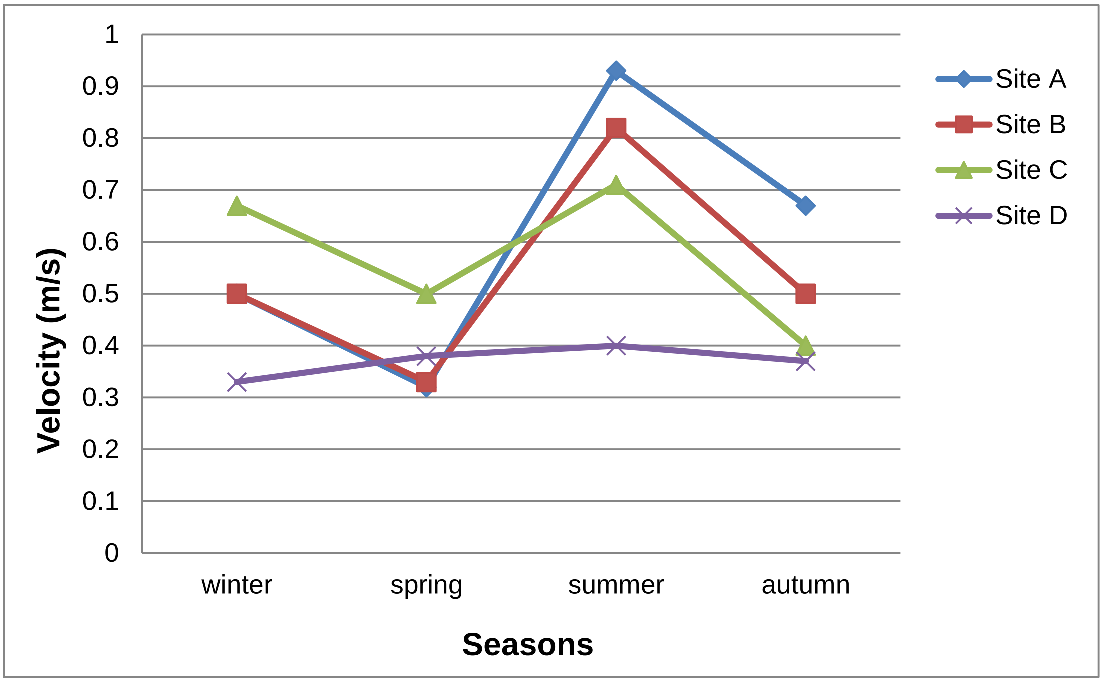

Figure 6: Variation of velocity at different sampling sites and seasons

4.2.2 Chemical parameters of water

4.2.2.1 Hydrogen ion concentration (pH)

The pH of the Kayar khola was noted to be slightly alkaline in all stations. During the summer season, site C recorded the lowest value of 7.6, while sites A and B recorded the maximum value of 8.5 respectively (figure 7).

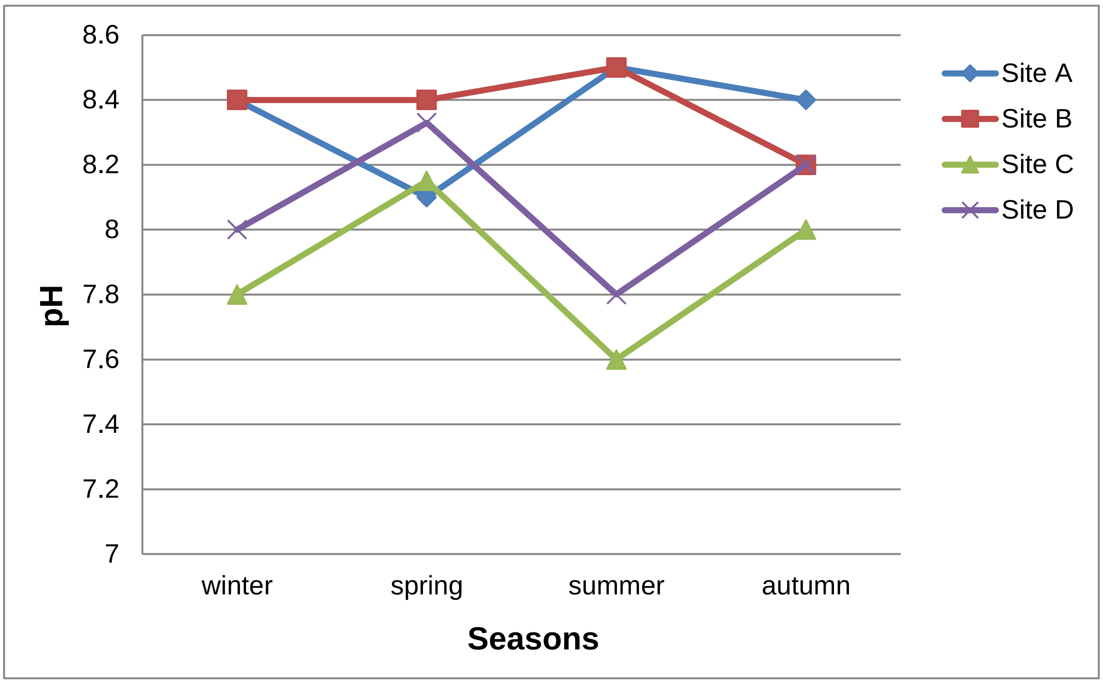

Figure 7: Variation of pH at different sampling sites and seasons

4.2.2.2 Dissolved oxygen (DO)

The highest dissolved oxygen concentration recorded at site A during the winter season was 10.2 mg/l. The minimum dissolved oxygen 6.1 mg/l was found at site C during summer season.

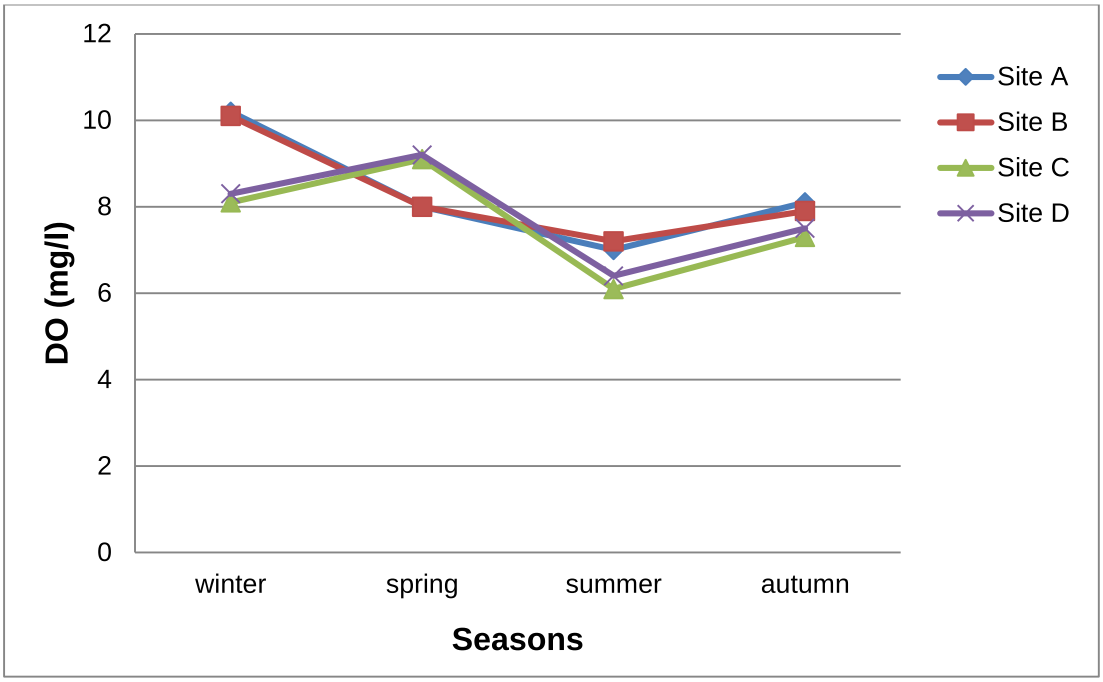

Figure 8: Variation of DO at different sampling sites and seasons

4.2.2.3 Free Carbon-dioxide (CO_2)

The highest value of free carbon-dioxide was recorded 2.4 mg/l at site C during summer season whereas the lowest value was 0.8 mg/l at site C and D in the spring season.

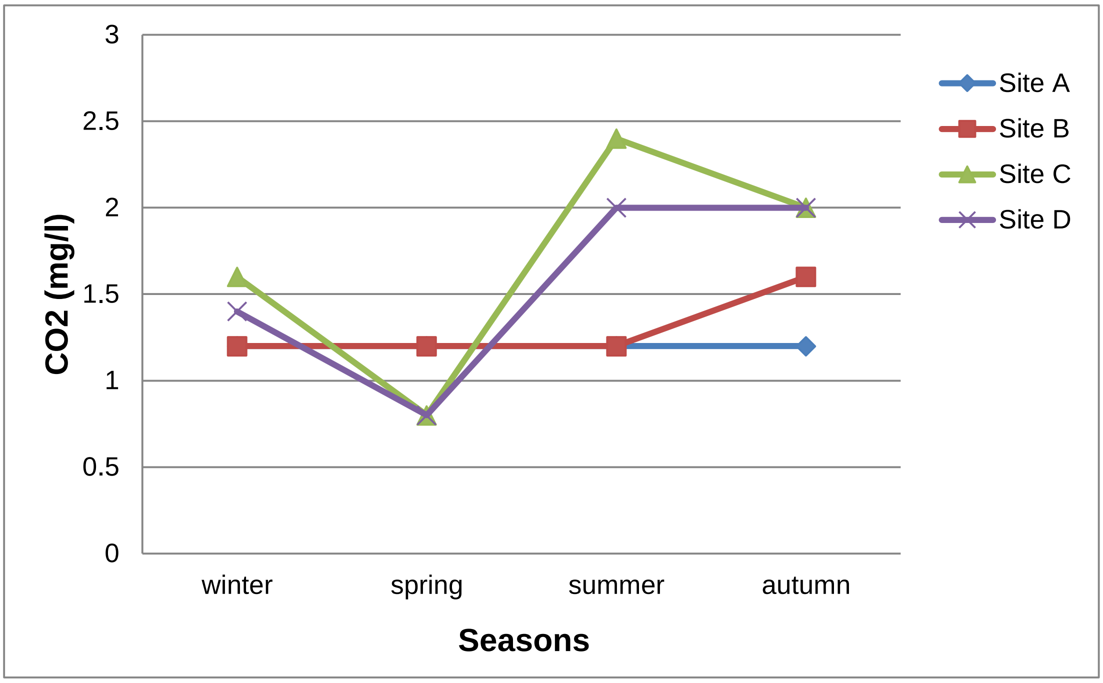

Figure 9: Variation of CO2 at different sampling sites and seasons

4.2.2.4 Total Hardness

The hardness of the water ranged from 212 mg/l to 294 mg/l. The total hardness of water was found to be highest at site A in spring season i.e., 294 mg/l and lowest hardness observed in the same site during summer season i.e., 212 mg/l.

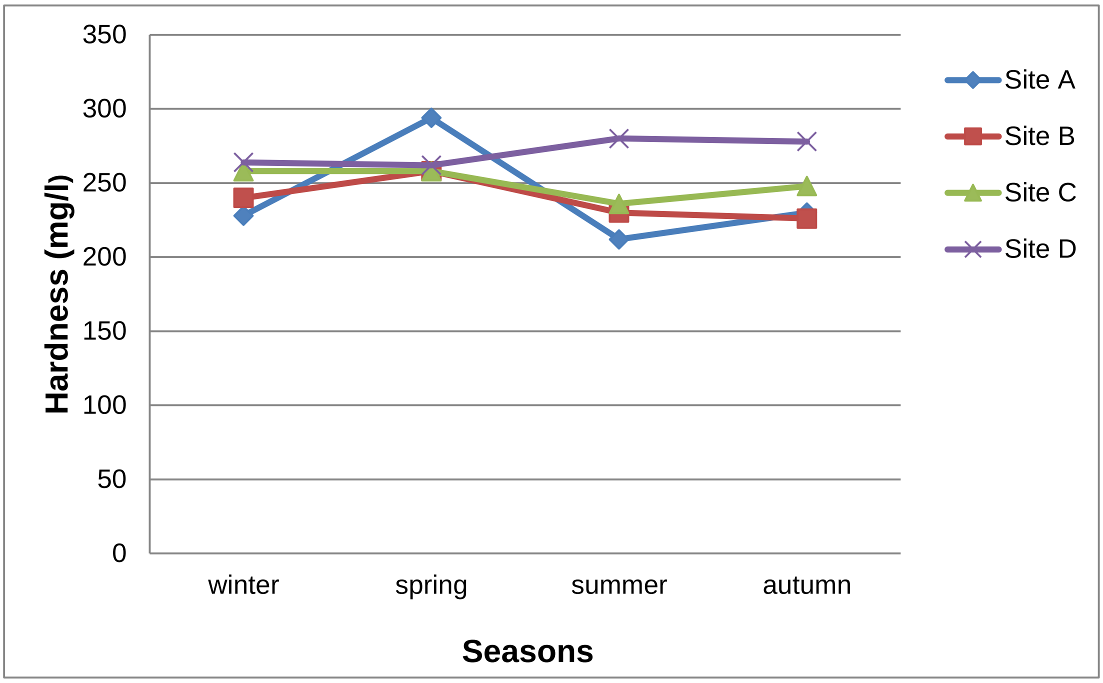

Figure 10: Variation of total hardness at different sampling sites and seasons

4.2.2.5 Alkalinity

The maximum alkalinity in water was recorded 238 mg/l at site C during spring season and the minimum was found 172 mg/l at site A in summer season.

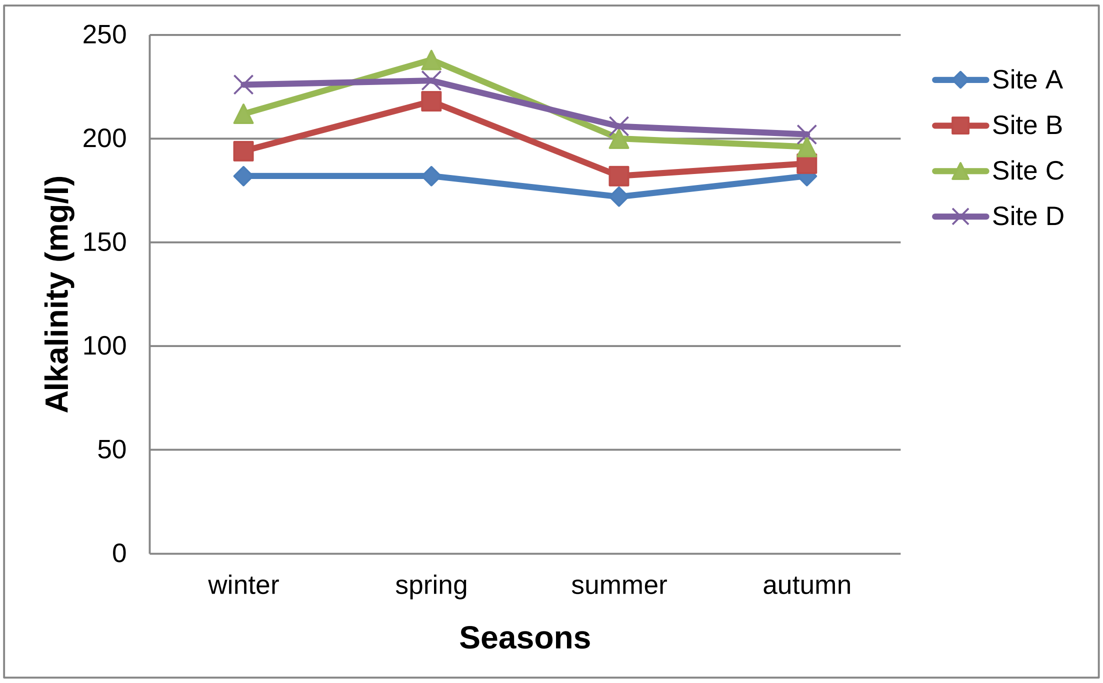

Figure 11: Variation of alkalinity at different sampling sites and seasons

4.3 Relation between macro-invertebrates and seasons

The relationship between macro-invertebrates and seasons were analyzed by using a one-way ANOVA (analysis of variance) test. There is a significant difference in abundance, Shannon index and family richness but no significant difference in evenness (Table 2).

Table 2: Results of analysis of variance (one-way ANOVA) test

	F value	**P value**
Abundance	25.51	< 0.05
Shannon index	5.14	< 0.05
Family richness	12.23	<0.05
Evenness	0.257	0.85

4.4 Ordination

The Eigen value of the first axis of Detrended Correspondence Analysis (DCA) was obtained 0.52 in the first DCA axis (Table 3). Thus Canonical Correspondence Analysis (CCA) was done.

Table 3: DCA summary

	DCA1	**DCA2**	**DCA3**	**DCA4**
Eigen values	0.52	0.18	0.05	0.09
Decorana values	0.57	0.11	0.03	0.01

4.4.1 Relationship between environmental variables and Macro-invertebrates.

The result obtained after the Canonical Correspondence analysis (CCA) was plotted in Figure 12. The vector length of a given variable indicates its importance in CCA analysis. In this analysis the longest vector length of pH at the second axis shows a highly significant relation with Ephemerellidae, Simuliidae, Psephenidae and Potamidae. Total hardness, alkalinity, free carbon-dioxide and depth were found to have a significantly correlated and strongly negative relation with pH, dissolved oxygen and water temperature. Occurrence of Families Viviparidae, Bulinidae, Thiaridae, Corduliidae, and Nepidae show positive correlation with free CO_2 and depth. Aeshnidae, Chlorocyphidae, Pleidae, Pachychilidae, and Libellulidae show a positive correlation with total hardness and alkalinity and strongly negative relation to temperature.

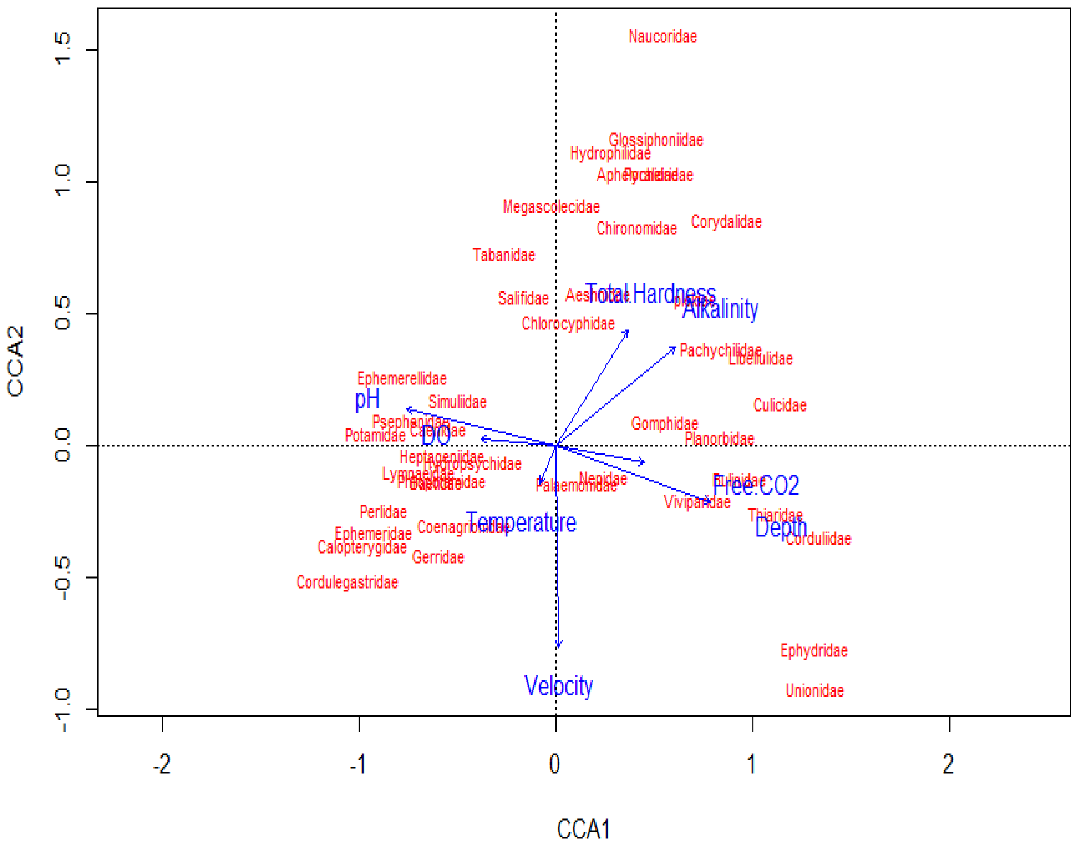

Figure 12: CCA analysis of macro-invertebrates abundance and environmental variables in the Kayar Khola. (DO=dissolved oxygen, free.CO2= free carbon-dioxide)

4.5 Cluster Analysis

4.5.1 Cluster analysis comparing macro-invertebrates taxa on the basis of macro- invertebrate assemblage

Hierarchical cluster dendrogram of macro-invertebrates from the Kayar Khola, black colored number represents the cluster number, red represents probability of Automatic Unbiased (AU) value and blue colored number represents Bootstrap Probability (BP) value. AU value > or = 95 represents a significant cluster.

There are altogether forty cluster groups formed with two major clusters 35 and 40 (Fig. 13). On the left side, 5, 14, 16, 22, 28, 32, 33, 36, 37 and 39 formed the significant cluster group. In cluster number 5, Libellulidae and Culicidae formed the significant cluster group with cluster number 16 with Corduliidae. Cluster number 37 delineated that Family Aeshnidae and Ephydridae formed the significant cluster. The cluster number 38 and 39 have no significant correlation between them. Similarly cluster number 35 delineated that the cluster number 30 and 31 have no significant correlation. In the right side, cluster number 9 shows that family Coenagrionidae and Cordulegastridae formed the significant cluster.

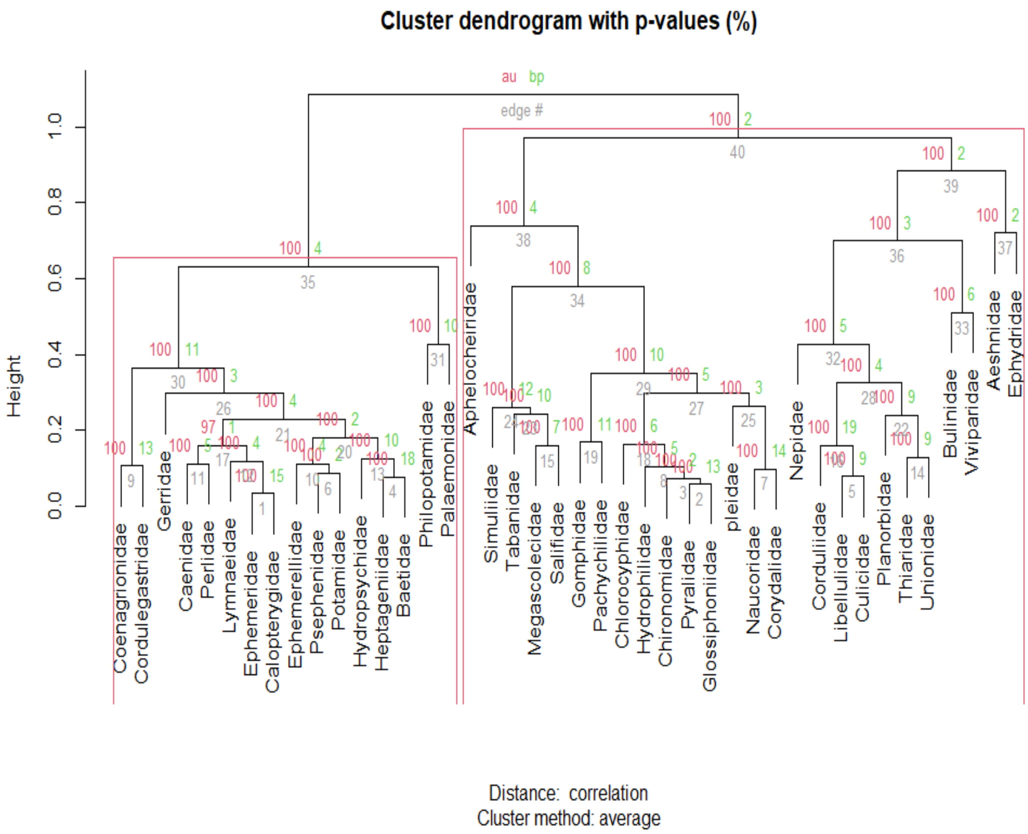

Figure 13: Dendrogram of cluster analysis comparing macro-invertebrates taxa on the basis of macro-invertebrate assemblage

4.5.2 Cluster analysis comparing sampling sites and seasons

The cluster dendrogram shows the relationship among sites and seasons on the basis of abundance of macro-invertebrates (Fig. 14). There are altogether fourteen cluster groups formed with two major clusters 11 and 14. Cluster number 4,8,12 and 16 form a significant cluster which shows the similar macro-invertebrates taxa from site D during four seasons i.e. winter, spring, summer and autumn. Similarly, cluster number 3,7,11 and 15 form a significant cluster group which shows the similar macro-invertebrates taxa from site C during four seasons. Cluster number 11 delineated that there is no relation between cluster number 6 and 8. The number 10 and 13 form a significant cluster between site B during summer season and site A during autumn season.

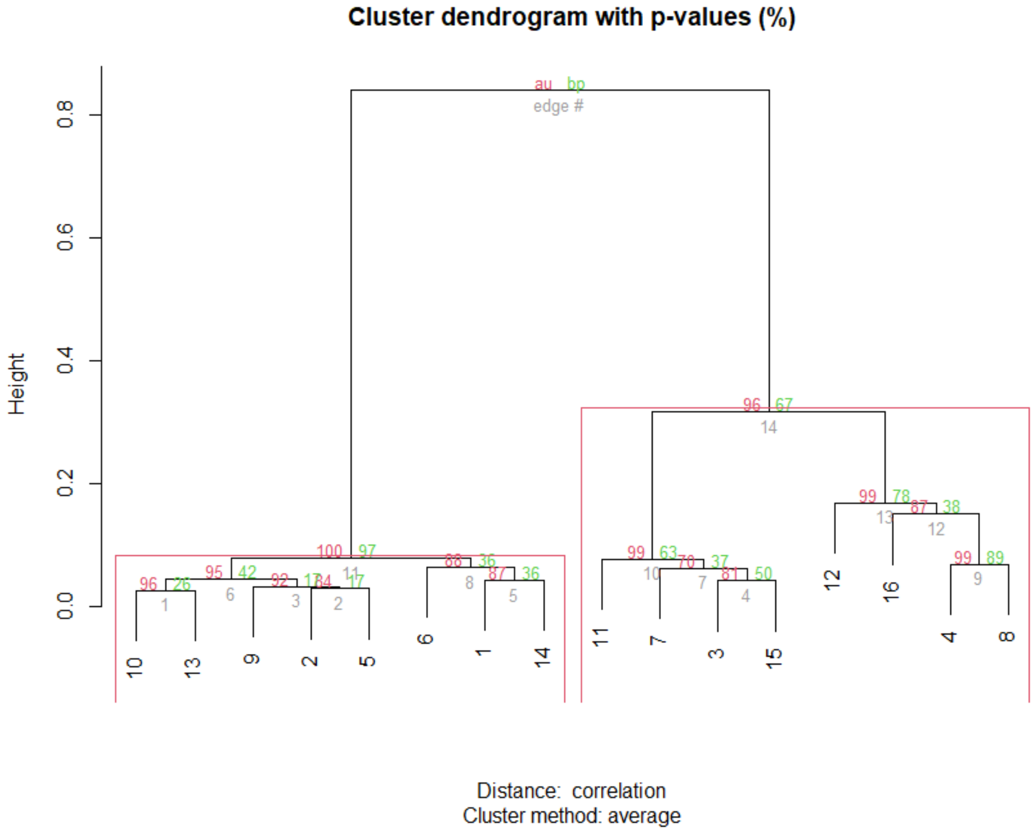

Figure 14: Dendrogram of cluster analysis comparing sampling sites and seasons on the basis of macro-invertebrate assemblage

Abbreviation: 1=Winter Site A, 2= Winter Site B, 3= Winter Site C, 4= Winter Site D, 5=Spring Site A, 6= Spring Site B, 7= Spring Site C, 8= Spring Site D, 9= Summer Site A, 10= Summer Site B, 11= Summer Site C, 12= Summer Site D, 13= Autumn Site A, 14= Autumn Site B, 15= Autumn Site C, 16= Autumn Site D.

5. DISCUSSION

The study was conducted for one year (November 2020 to August 2021) covering four seasons. The main goal of the study was to explore the aquatic macro-invertebrates fauna, their diversity and their relation with the environmental factors of the Kayar Khola. Altogether 2005 individual aquatic macro-invertebrates (mostly aquatic insects and mollusks) of 42 Families were collected from four sampling sites at Kayar Khola during four seasons. They belong to 17 Orders, 5 Classes and 3 Phyla. There has been no investigation of the diversity and distribution of macro-invertebrates in Kayar Khola. So this study was baseline for further study.

Among 42 Families, Hydropsychidae (20.20%) was dominated followed by Thiaridae (15.76%), Heptageniidae (12.02%), Baetidae (9.43%), and Psephenidae (5.09%). In our study Order Trichoptera was highly abundant. Similar result was found by Sharma (1999). He observed higher abundance of Trichoptera followed by Coleoptera in Saptakoshi River. After Tricoptera Ephemeroptera was a diverse group found in the Kayar Khola. In consistent with our study Khatri et al. (2022) found Hydropsychidae and Baetidae were most abundant taxa in the Bheri River system.

Trichoptera consist of two Families ; Hydropsychidae and Philopotamidae with one identified Genera *Hydropsyche* sp. Ephemeroptera consist of five Families ; Heptageniidae, Baetidae, Ephemeridae, Ephemerellidae and Caenidae with four identified Genera namely *Heptagenia* sp., *Baetis* sp., *Serratella* sp., *Caenis* sp. Coleoptera consist of two Families ; Psephenidae and Hydrophilidae. Odonata consist of eight Families with only one identified Genera *Ophiogomphus* sp. Hemiptera consist of five Families; Nepidae, Gerridae, Pleidae, Aphelocheiridae, Naucoridae with one identified Genera *Aphelocheirus* sp. Diptera consist of five Families with two Genera namely *Tabanus* sp. and *Simulium* sp. Plecoptera consist of only one Family Perlidae with single Genera *Neoperla* sp. Similarly Megaloptera consist of one Family Corydalidae and a genus *Corydalus* sp. Lepidoptera consist of single Family Pyralidae with a genus *Aulocodes* sp. Caenogastropoda consist of two Families ; Thiaridae and Pachychilidae with four species namely *Tarebia granifera* Lamarck, 1822, *Tarebia lineata* Gray, 1828, *Melanoides tuberculata* Mueller, 1774 and *Brotia costula* Rafinesque, 1833. Family Lymnaeidae consist of single species *Lymnaea acuminata*

Lamarck, 1822, Planorbidae consist of one species *Gyraulus convexiusculus* Hutton, 1849, Bulinidae consist of single species *Indoplanorbis exustus* Deshayes, 1834 which belong to the Order Basommatophora and class Gastropoda. Order Mesogastropoda consists of the Viviparidae Family with a single species *Bellamy bengalensis* Lamarck, 1822. Unionoida consists of the Unionidae Family with two Genera *Parreysia* sp. and *Radiatula* sp. They belong to the Class Bivalvia.

Family Ephemeridae, Philopotamidae, Calopterygidae, Perlidae and Potamidae were recorded from site A and B. It might be due to the favorable environmental conditions at these locations. Corduliidae, Libellulidae, Culicidae, Thiaridae, Pachychilidae and Bulinidae were found from Site C and D. Psephenidae was not found in site C and Pleidae was not found in site A throughout the study period. It might be due to the existence of stagnant water at site C. Similar result was found by Raphahlelo et al. (2022) in the Mohlabetsi River. They observed structural composition of macro-invertebrates varied among the sites. Heptageniidae, Baetidae, Caenidae, Hydropsychidae, Chlorocyphidae were briefly recorded from all four sampling sites. Similar result was obtained from Bhandari et al. (2018). They found Ephemeroptera, Trichoptera, Coleoptera, Diptera and Odonata are habitat generalists and they are present in all habitat types. Hydrophilidae, Tabanidae, Simuliidae, Megascolecidae and Salifidae were found from the sites A and D. Cordulegastridae found only from the sita A which comprised 0.20% of total collection. Similarly, Aphelocheiridae, Naucoridae, Corydalidae, Pyralidae and Glossiphoniidae were recorded only from site D. Gomphidae, Nepidae, Gerridae, Chironomidae and Palaemonidae were also recorded from all sites whereas Ephydridae, Viviparidae and Unionidae were found only from site C.

Most of the macro-invertebrates were recorded from all four seasons. The highest number of individuals were recorded in the winter whereas the least recorded in the summer. Similar result was found by Sharma et al. (2004). They observed higher taxa richness and population density during the winter and lower during the summer in various lotic water-bodies. Heptageniidae, Baetidae, Ephemeridae, Hydropsychidae, Psephenidae, Gomphidae, Libellulidae, Nepidae, Gerridae, Pleidae, Simuliidae, Chironomidae, Perlidae, Palaemonidae, Thiaridae and Unionidae were found in all four seasons; winter, spring, summer and autumn. Calopterygidae reported only in the winter season which comprised 0.20% of total collection. Ephemerellidae, Caenidae, Hydrophilidae, Chlorocyphidae, Tabanidae, Potamidae, Salifidae and Planorbidae were recorded from winter, spring and autumn. Aeshnidae, Aphelocheiridae and Viviparidae were found in spring and autumn but not in winter and summer. Ephydridae was recorded from summer and autumn whereas Pyralidae, Glossiphoniidae and Pachychilidae were found in spring and autumn season.

In this study, the maximum diversity index value (2.46) was found in site D, and 2.39 in the winter season. The lowest value (1.88) was obtained in site C, and 1.61 was observed during the summer season. In consistency with our observation, Barman & Gupta (2015) reported a high diversity index in the pre-monsoon season and a comparatively low diversity index in the post-monsoon season from Bakuamari stream, Chakrashila Wildlife Sanctuary, Assam. Similarly maximum family richness value 18.75 was observed at site D and 21 in winter season. The minimum value 12.75 was found at station B and 7.75 during summer season. Similar result was found by Brewin et al. (2000). In several lotic water-bodies, taxa richness and population density were higher in autumn/winter and lower in summer/monsoon season. The maximum Evenness index (0.87) was found at station D and 0.79 in winter and summer season. Similarly lowest (0.72) was found at station C and 0.76 during spring season. The maximum value suggests that aquatic macro-invertebrates are homogeneous, whereas the lowest value implies that aquatic macro-invertebrates are heterogeneous in a given area.

The quality of water was determined by various physicochemical factors like temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and total hardness and these are useful to detect pollution levels in the water ecosystem. Changes in water trophic conditions are reflected in the biological community structure, which includes species pattern, distribution, and diversity (Fouzia & Amir 2013). Temperature is one of the major factors that alter the functions of the aquatic ecosystem, and it affects the growth and distribution of flora and fauna (Tank & Chippa 2013). The temperature of Kayar Khola was recorded high in the summer season and low in the winter season. Similar type of finding was outlined by Barman & Gupta (2015). They observed a maximum water temperature in the pre monsoon than in the post monsoon season. The coverage and variability of the vegetation on the river side in each study site induced changes in water temperature in the study area. In contradiction with our result, Melo & Froehlich (2001) observed minor temperature differences across all sites.

The abundance and diversity of macro-invertebrates were affected by velocity. This is because high flow events diminish biomass and alter the species composition of invertebrates in aquatic ecosystems (Barman & Gupta 2015). Maximum velocity was found in summer season and minimum found in spring season in Kayar Khola.

pH has an impact on aquatic species because the majority of their metabolic activities are pH dependent (Patel & Parikh 2013). According to WHO water with a pH value of 6.5 to 8.5 is considered normal. The pH of acidic water is less than 7, whereas the pH of basic water is greater than 7. The pH of the Kayar khola was found to be alkaline throughout the year in the current study, i.e. greater than 7 pH. It ranged from 7.6 to 8.5.

DO is essential for survival of aquatic organisms and is also used to determine the freshness of a river (Fakayode 2005). DO concentration in natural water is affected by physical, chemical, and biological activity in the water body (Kazi et al. 2009). DO decreases as water temperature increases, hence warmer water results in low DO (Mandal 2014). In this study, dissolved oxygen was found to be negatively correlated with water temperature. Similar result was obtained by Gautam & Bhattarai (2008). In our study, maximum DO concentration occurs in winter season whereas minimum in summer season. The DO levels below 5 mg/l cause stress to aquatic life but our result obtained between 6.1 mg/l to 10.2 mg/l. Thus Kayar khola is suitable for aquatic life.

The production of free carbon-dioxide is dependent on respiration of biota, photosynthesis, and decomposition (Choudary et al. 2014). The increase in CO_2 levels in freshwater causes weak acidification, which is unsuitable for certain macro-invertebrate species. The high value of CO_2 is observed in the summer season while low in the spring season. The higher the value of CO_2 may be attributed to a decline in production, which causes decomposition and the formation of more CO_2 in water (Rajan & Samuel 2016).

Total hardness is the water quality parameter that describes the effect of dissolved minerals (mainly Ca & Mg) determining the suitability of water for household, industrial and drinking purposes (Taylor 1949). It is measured in calcium carbonate (mg/l).

According to WHO (2003), the range between 0-40 mg/l for soft, 40-100 mg/l for moderately hard, and 100-300 mg/l for extremely hard. In Kayar Khola, the highest amount of total hardness i.e. 294 mg/l is found at site A during spring and lowest 212 mg/l observed in the same site during summer. It might be due to temperature variation and chemical reaction in water.

The ability of water to resist acidification is referred to as alkalinity. Freshwater environments commonly have alkalinity levels ranging from 20 to 200 mg/L. Alkalinity levels less than 10 mg/L suggest a poorly buffered water system (Clausen & Biggs 1997). The alkalinity level in Kayar Khola was found to be 172-238 mg/l which was common in the freshwater ecosystem. The spring season had the highest alkalinity, which could be due to less water volume and more bi-carbonates.

Shah et al. (2020) found taxa richness and abundance did not differ between the pre monsoon and post monsoon seasons, within and between watersheds. But our result was contradictory to their study. The analysis of variance indicated a significant difference in abundance, Shannon index and family richness ($P < 0.05$) but no significant difference in evenness ($P > 0.05$). This is because of variation in habitat, and diverse flow types. Consistent with our study Shah et al. (2011) found a significant difference between the seasons from Jagdishpur reservoir.

There are altogether fourteen cluster groups formed among the sites and seasons. Those clusters found to have similar habitat types are significantly correlated to each other. Similar types of individuals were found on that cluster. The similarity between macro-invertebrates decreases as the distance of the cluster increases. Similar result was observed by Raphahlelo et al. (2022). They found different individual species at different cluster groups.

The ordination plot revealed that total hardness, alkalinity, free CO_2, and depth were found to have a significantly correlated and strongly negative relation with pH, dissolve oxygen and water temperature. Similar type of result was observed by Keke et al. (2017). They found highly significant relationships between species abundances and environmental parameters. High values of total hardness and alkalinity were associated with Families like Aeshnidae, Chlorocyphidae, Pleidae, Pachychilidae, and Libellulidae. In our study, the longest vector length of pH at the second axis shows a significant relation with Ephemerellidae, Simuliidae, Psephenidae and Potamidae. It means that these Families were highly affected by pH.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

A total of 2005 macro-invertebrate individuals belonging to 17 Orders and 42 Families were found in Kayar Khola. The Hydropsychidae Family had the highest frequency across all sites and seasons, whereas the Viviparidae had the lowest frequency which was only found in spring season at site C of Kayar Khola. The results in the present study showed that spatial and temporal variations in macro-invertebrate diversity were significantly correlated to physico-chemical parameters such as water temperature, pH, dissolved oxygen, free carbon-dioxide, hardness and alkalinity of the water. By analyzing those parameters it can be concluded that the quality of water in Kayar Khola was in good condition and was suitable for macro-invertebrates and other aquatic organisms.

6.2 Recommendations

Based on this research, some important recommendations are given below:

- The identification of macro-invertebrates up to Species level is required.
- The relationship between macro-invertebrates and environmental factors such as climate, substrate composition, macrophytes, acidity, turbidity, biological oxygen demand, chemical oxygen demand should be analysed.
- Variety of collection techniques can be used when sampling macro-invertebrates.

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ANNEXES

ANNEXES I Photo Plates of collected species

Gomphidae

Perlidae

Culicidae

Naucoridae

Simuliidae

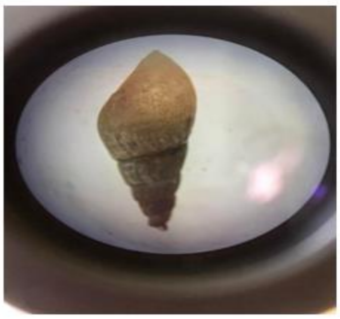
Hydropsychidae

Ephemerellidae

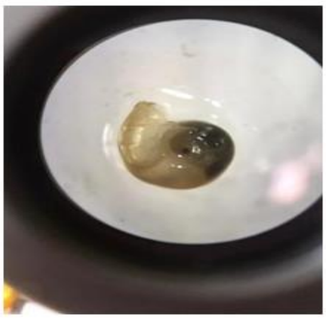
Tabanidae

Psephenidae

Megascolecidae



Thiaridae



Bulinidae

ANNEXES II Photo Plates of sampling sites and research activities

Site A: Shaktikhor

Site B: Jutpani

Site C: Belchi

Site D: Sauraha

Collection of macro-invertebrates

Titration of water sample in lab

ANNEXEX III Distributional pattern and frequency occurrence of macro-invertebrates

	winter				**spring**				**summer**				**autumn**					
	site A	site B	site C	site D	site A	site B	site C	sit e D	site A	site B	site C	site D	site A	site B	site C	site D	Total	frequenc y
Heptageniidae	40	35	8	12	38	20	5	8	12	9	0	2	30	15	0	7	241	12.02
Baetidae	35	22	6	7	20	15	6	5	8	12	0	2	26	20	0	5	189	9.43
Ephemeridae	12	6	0	0	2	1	0	0	1	1	0	0	2	1	0	0	26	1.30
Ephemerellidae	8	5	0	0	7	8	0	1	0	0	0	0	2	5	0	0	36	1.80
Caenidae	6	5	0	1	3	2	2	0	0	0	0	0	1	0	0	0	20	1.00
Hydropsychidae	60	52	25	10	45	50	20	10	20	15	3	0	40	30	20	5	405	20.20
Philopotamidae	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0.15
Psephenidae	30	11	0	6	12	15	0	5	2	3	0	0	8	10	0	0	102	5.09
Hydrophilidae	0	0	0	6	2	0	0	3	0	0	0	0	0	0	0	2	13	0.65
Chlorocyphidae	4	3	2	13	0	0	2	6	0	0	0	0	4	3	0	1	38	1.90
Gomphidae	4	3	10	13	2	2	3	5	0	0	3	4	1	2	3	3	58	2.89
Corduliidae	0	0	22	2	0	0	0	0	0	0	0	0	0	0	5	3	32	1.60
Libellulidae	0	0	21	12	0	0	0	6	0	0	1	1	0	0	20	12	73	3.64
Coenagrionidae	4	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	8	0.40

Cordulegastridae	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	4	0.20
Calopterygidae	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0.20
Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	3	0	2	3	8	0.40
Nepidae	3	2	5	2	3	1	3	2	0	1	0	1	2	0	3	0	28	1.40
Gerridae	3	1	0	0	2	0	0	1	0	0	1	0	2	1	0	0	11	0.55
pleidae	0	7	8	18	0	0	7	10	0	0	0	5	0	0	7	12	74	3.69
Aphelocheiridae	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	3	0.15
Naucoridae	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	3	0.15
Tabanidae	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	4	0.20
Simuliidae	15	0	0	11	4	0	0	2	1	0	0	0	3	0	0	0	36	1.80
Culicidae	0	0	4	2	0	0	0	0	0	0	0	0	0	0	2	1	9	0.45
Chironomidae	3	2	0	23	0	0	0	15	0	0	0	3	2	2	4	8	62	3.09
Ephydridae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	3	0.15
Perlidae	3	2	0	0	1	1	0	0	1	0	0	0	1	0	0	0	9	0.45
Corydalidae	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	1	4	0.20
Pyralidae	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	2	0.10
Palaemonidae	6	9	5	3	2	4	3	0	0	0	3	0	2	0	4	0	41	2.04
Potamidae	3	1	0	0	2	1	0	0	0	0	0	0	0	1	0	0	8	0.40
Glossiphoniidae	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	3	0.15

Megascolecidae	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	3	0.15
Salifidae	2	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	5	0.25
Thiaridae	0	0	112	25	0	0	62	22	0	0	17	8	0	0	52	18	316	15.76
Pachychilidae	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	3	0.15
Lymnaeidae	30	10	5	0	4	10	2	0	0	0	0	0	7	12	0	0	80	3.99
Planorbidae	0	0	3	0	0	2	3	0	0	0	0	0	0	0	2	0	10	0.50
Bulinidae	0	0	2	0	0	0	2	1	0	0	1	0	0	0	0	2	8	0.40
Viviparidae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.05
Unionidae	0	0	7	0	0	0	3	0	0	0	4	0	0	0	5	0	19	0.95
	279	179	246	177	150	132	124	108	45	41	34	27	140	102	133	88	2005	100.00